A Novel Classification of MUC1 Expression Is Correlated with Tumor Differentiation and Postoperative Prognosis in Non–Small Cell Lung Cancer

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Background: MUC1 is a transmembrane mucin that plays an important role in tumor progression. Many clinical studies have suggested that the expression pattern of MUC1 core protein can be a useful prognostic marker in various malignancies, but the prognostic significance in non–small cell lung cancer (NSCLC) remains uncertain. We performed a study to assess clinical significance, especially prognostic impact, of MUC1 expression in NSCLC.

Methods: A total of 62 patients with completely resected pathologic stage I to IIIA NSCLC were retrospectively reviewed. Histologic sections cut from primary tumors were immunohistochemically stained with an anti-MUC1 monoclonal antibody (CA15-3, clone DF3), which recognizes unglycosylated epitope of MUC1 core protein. According to MUC1 expression pattern, each patient was classified into the high-grade polarized expression (HP), the low-grade polarized expression (LP), or the depolarized expression (D) group.

Results: Twenty-four (38.7%), 21 (33.9%), and 17 (27.4%) patients were classified into the HP group, the LP group, and the D group, respectively. HP was exclusively seen in adenocarcinoma, mostly in well-differentiated adenocarcinoma. D was correlated with progressive stage and lymph node metastasis. Postoperative survival of the D group seemed to be poorer than that of the HP group for all NSCLC patients, and the difference was enhanced in adenocarcinoma patients.

Conclusion: A novel classification of MUC1 expression pattern (HP, LP, and D) was correlated with tumor differentiation and postoperative survival in NSCLC, especially in lung adenocarcinoma.

Key Words: MUC1, Lung cancer, Prognosis, Immunohistochemistry.

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Primary lung cancer is the leading cause of death from cancer in most industrialized countries, and non–small cell lung cancer (NSCLC) accounts for 70% to 80% of primary lung cancer. Although some improvements in surgery, chemotherapy, and radiotherapy have been made recently, the prognosis of NSCLC patients remains unsatisfactory. The most important reason for the poor prognosis is the high incidence of nodal and/or distant metastases. To improve the prognosis, it is essential to reveal biological characteristics leading to development of metastasis in NSCLC.

MUC1 is a transmembrane mucin consisting of a heavily *O*-glycosylated extracellular domain, a transmembrane domain, and a cytoplasmic tail of 72 amino acids; the extracellular domain has a variable number of highly conserved tandem repeats of 20 amino acids.[1–3] In normal tissues, including bronchial mucosa and type II pneumocytes, MUC1 is expressed on the apical surface of normal glandular epithelial cells.[4–6] In neoplastic tissues, MUC1 expression may be up-regulated, or the glycosylation may be altered and MUC1 may be expressed on the entire cell surface (depolarized expression).[4,7] Experimental studies have shown that MUC1 may reduce cell–cell and cell–matrix adhesion,[8,9] and the cytoplasmic domain of MUC1 interacts with a variety of molecules involved in tumor proliferation such as epidermal growth factor receptor, c-Src, β-catenin, and Grb2,[10–13] and that MUC1 overexpression is correlated with tumorigenesis in a mouse model.[14] These studies have suggested that MUC1 plays important roles in development and progression of malignant tumors.

MUC1 is also recognized as a target molecule in immunotherapy for various malignant tumors, because unmasked epitopes of MUC1 core protein expressed on tumor cells can elicit a strong antitumor immunity.[15–18] When we select candidates for MUC1-targeted immunotherapy, it is important to evaluate MUC1 expression on the surface of tumor cells.

It has been reported that MUC1 is an important prognostic factor in various malignant tumors.[19–30] These studies have suggested that alteration in the expression pattern of MUC1 is important in evaluation of clinical significance of MUC1. In NSCLC, however, the clinical significance including prognostic impact of MUC1 expression status remains uncertain.[6,31,32] Thus, we conducted a detailed study on MUC1 expression,

including the cellular distribution of MUC1, in correlation with clinical outcomes in NSCLC, and reported a significant correlation between a novel classification of MUC1 expression pattern and histologic differentiation and postoperative survival.

PATIENTS AND METHODS

Patients and Tissue Preparation

A total of 63 consecutive patients with pathologic stage I to IIIA NSCLC, who underwent complete tumor resection and mediastinal lymph node dissection without any preoperative therapy at the Kyoto University Hospital between July of 1996 and October of 1998 and from whom written informed consent for the research use of resected tumor tissues were taken, were retrospectively reviewed (Table 1). Complete tumor resection was considered achieved when microscopic cancer cells were identified neither in the margin of resection of the tumor nor in the highest mediastinal lymph nodes.[33] One patient was excluded from the study because of operation-related death, and thus a final total of 62 patients were evaluated. Pathologic stage was evaluated according to the current tumor, node, metastasis classification as revised in 1997.[34] Histologic type and tumor cell differentiation were determined using the current classification by the World Health Organization as revised in 2004.[35] For analyses according to the differentiation of cancer cells, well-differentiated squamous cell carcinoma and adenocarcinoma were classified as well-differentiated tumors and moderately differentiated squamous cell carcinoma and adenocarcinoma as moderately differentiated tumors; large-cell carcinoma and poorly differentiated squamous cell carcinoma and adenocarcinoma were classified as poorly differentiated tumors. For all these patients, records of surgery, inpatient medical records, chest radiography films, whole-body computed tomography films, and bone scanning films were reviewed without knowledge of the results of the IHS. As postoperative adjuvant therapy, oral administration of UFT (tegafur and uracil) was prescribed for patients with pathologic stage I to IIB, and cisplatin based chemotherapy was prescribed for patients with pathologic stage IIIA. Follow-up of postoperative clinical course was conducted by outpatient medical records and by inquiries by telephone or letter.

Histologic specimens were available for immunohistochemical staining (IHS) from all patients. Tumor specimens were immediately fixed in 10% (v/v) formalin, and then embedded in paraffin. Serial 4-μm sections were prepared from each primary tumor sample and were served for hematoxylin and eosin staining and IHS for evaluation of MUC1 expression. Slides were reviewed independently by two investigators (S.N. and K.T.) without knowledge of any clinical data. A different evaluation of MUC1 expression was made in seven patients (11.3%). The field was reevaluated until the evaluation coincided. This study was reviewed and approved by the Ethics Committee of the Graduate School and Faculty of Medicine, Kyoto University.

TABLE 1. Patient Characteristics and MUC1 Expression in Resected NSCLC

	No. of Patients (%)	HP (%)	LP (%)	D (%)	*p* Value
Total	62 (100)	24 (38.7)	21 (33.9)	17 (27.4)	
Mean age (yr)		63.5	63.7	66.3	0.643
<65	35 (56.5)	12 (34.3)	11 (31.4)	12 (34.3)	0.381
≥65	27 (43.5)	12 (44.4)	10 (37.0)	5 (18.5)	
Gender					
Male	41 (66.1)	10 (24.4)	16 (39.0)	15 (36.6)	0.004
Female	21 (33.9)	14 (66.7)	5 (23.8)	2 (9.5)	
Histologic type					
Adenocarcinoma	47 (75.8)	24 (51.1)	17 (36.2)	6 (12.8)	<0.0001*
Squamous cell	11 (17.7)	0	1 (9.1)	10 (90.9)	
Large-cell	4 (6.5)	0	3 (75.0)	1 (25.0)	
Tumor differentiation					
Well	30 (48.4)	21 (70.0)	7 (23.3)	2 (6.7)	<0.0001
Moderate/poor	32 (51.6)	3 (9.4)	14 (43.8)	15 (46.9)	
Pathologic stage					
I	44 (71.7)	22 (50.0)	15 (34.1)	7 (15.9)	0.002
II/IIIA	18 (29.3)	2 (11.1)	6 (33.3)	10 (55.6)	
Pathologic T factor					
T1	30 (48.4)	16 (53.3)	11 (36.7)	3 (10.0)	0.008
T2/T3	32 (51.6)	8 (25.0)	10 (31.3)	14 (43.8)	
Pathologic N factor					
N0	47 (75.8)	22 (46.8)	16 (34.0)	9 (19.1)	0.017
N1/N2	15 (24.2)	2 (13.3)	5 (33.3)	8 (53.3)	

*Comparison between adenocarcinoma and squamous cell carcinoma plus large-cell carcinoma. HP, high-grade polarized expression; LP, low-grade polarized expression; D, depolarized expression.

Immunohistochemical Staining

Expression of MUC1 was evaluated with IHS using a streptavidin-biotinylated horseradish peroxidase detection system (LSAB+ kit/HRP; DAKO, Kyoto, Japan). Sections were incubated overnight at 4°C with an anti-MUC1 mouse monoclonal antibody (CA15-3, clone DF3; DAKO) diluted at 1:50 without antigen retrieval; for negative control slides, the primary antibody was omitted. As a chromogen, diaminobenzidine-tetrahydrochloride (0.03%) containing 0.1% hydrogen peroxide was used, and sections were counterstained with hematoxylin.

Evaluation of Immunohistochemical Analysis

Staining intensity of MUC1 expression was first classified into negative or positive, and then, when positive, each tumor cell was further classified according to the expression pattern into polarized or depolarized expression as follows: (1) polarized expression when MUC1 is localized in the cell membrane of apical portion of tumor cells (Figure 1 *A*), (2) depolarized expression when MUC1 is observed over the entire cell surface or whole cytoplasm (Figure 1 *B*). According to the percentage of tumor cells showing polarized MUC1 expression and that with depolarized MUC1 expression, MUC1 expression status of each patient was finally divided into the high-grade polarized (HP), the low-grade polarized (LP), or the depolarized (D) group (Table 2). According to the definition, a patient with tumor showing no MUC1 expression was classified into the LP group.

Statistical Analysis

Counts were compared by means of the χ^2. Continuous data were compared using the Student's *t* test if the sample distribution was normal or using the Mann-Whitney *U* test if the sample distribution was asymmetrical. The postoperative overall survival rate was analyzed by the Kaplan-Meier method, and the differences in survival rates were assessed by the log rank test and the generalized Wilcoxon's test. Multivariate analysis of prognostic factors was performed using the Cox's regression model. Differences were considered significant when $p < 0.05$. All statistical manipulations were performed using the StatView software system for Macintosh (Version 4.5; Abacus Concepts Inc., Berkeley, CA).

RESULTS

MUC1 Expression and Clinicopathologic Features

Of all 62 patients, 24 (38.7%), 21 (33.9%), and 17 (27.4%) were classified as the HP, LP, and D group, respectively. Clinicopathologic features according to MUC1 status are summarized in Table 1. Male patients were less frequently classified into the HP group, and more frequently were classified into the D group ($p = 0.004$). Well-differentiated tumor and early-stage patients were more frequently classified into the HP group; moderately to poorly differentiated tumor and advanced-stage patients were more frequently into the D group.

The most significant was the correlation between MUC1 status and histologic type; most squamous cell carcinoma patients were classified into the D group, and no squamous cell carcinoma or large-cell carcinoma patient was into the HP group. As adenocarcinoma patients were distributed into the three groups to some degree, an analysis only in adenocarcinoma patients were further performed (Table 3). A significant correlation between tumor differentiation and MUC1 status was observed; no well-differentiated tumor patient was classified into the D group, and most well-differentiated tumor patients including two bronchioalveolar carcinoma patients were classified into the HP group. Because 17 (89.5%) of the 19 female adenocarcinoma patients had well-differentiated tumors, the female patients might be more frequently classified into the HP group. There was no significant correlation between MUC1 status and age, pathologic stage, pathologic T factor, or pathologic N factor.

MUC1 Status and Postoperative Survival

For all patients, 5-year survival rates of the HP, LP, and D patients were 78.3%, 51.5%, and 56.2%, respectively, showing a trend of a favorable prognosis in HP patients and a poor prognosis in D patients ($p = 0.134$ by the log rank test and $p = 0.081$ by the Wilcoxon test among the three groups) (Figure 2); the *p* values between HP and D patients by the log rank test and the Wilcoxon test were 0.074 and 0.042, respectively (Figure 2).

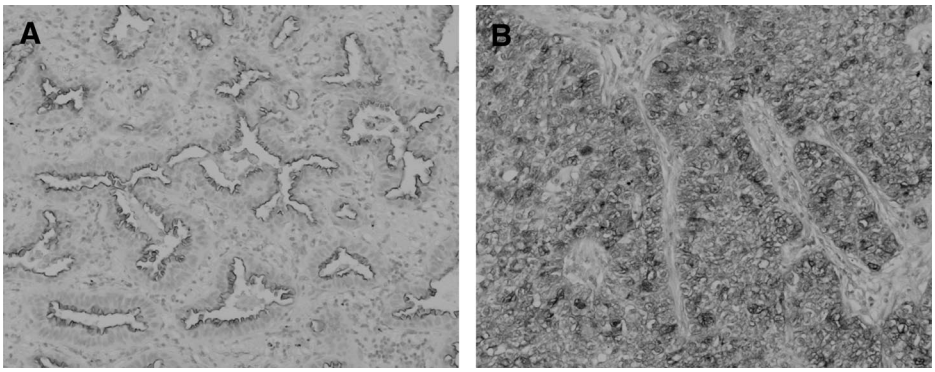

FIGURE 1. MUCI expression in NSCLC with immunohistochemical staining. (*A*) High-grade polarized expression (HP) pattern of MUC1 expression in a well-differentiated papillary adenocarcinoma case (original magnification, ×100). (*B*) Depolarized (D) expression pattern of MUC1 in a poorly differentiated adenocarcinoma case (original magnification, ×200).

TABLE 2. Classification of MUC1 Expression Status

	Classification of MUC1 Expression Status		
	HP	**LP**	**D**
Percentage of tumor cells showing polarized MUC1 expression	≥50%	<50%	Any
Percentage of tumor cells showing depolarized MUC1 expression	<10%	<10%	≥10% or more

TABLE 3. Characteristics of Patients and MUC1 Expression in Resected Lung Adenocarcinoma

	No. of Patients (%)	**HP (%)**	**LP (%)**	**D (%)**	***p* Value**
All patients	47 (100)	24 (51.1)	17 (36.2)	6 (12.8)	
Mean age (yr)		63.5	66.6	65.2	0.596
<65	27 (57.4)	12 (44.4)	11 (40.7)	4 (14.8)	0.571
≥65	20 (42.6)	12 (60.0)	6 (30.0)	2 (10.0)	
Gender					
Male	28 (59.6)	10 (35.7)	12 (42.9)	6 (21.4)	0.017
Female	19 (40.4)	14 (73.7)	5 (26.3)	0	
Tumor differentiation					
Well	28 (59.6)	21 (75.0)	7 (25.0)	0	<0.0001
Moderate/poor	19 (40.4)	3 (15.8)	10 (52.6)	6 (31.6)	
Pathologic stage					
I	40 (85.1)	22 (55.0)	14 (35.0)	4 (10.0)	0.283
II/IIIA	7 (14.9)	2 (28.6)	3 (42.9)	2 (28.6)	
Pathologic T factor					
T1	29 (61.7)	16 (55.2)	11 (37.9)	2 (6.9)	0.308
T2/T3	18 (38.3)	8 (44.4)	6 (33.3)	4 (22.2)	
Pathologic N factor					
N0	40 (85.1)	22 (55.0)	14 (35.0)	4 (10.0)	0.283
N1/N2	7 (14.9)	2 (28.6)	3 (42.9)	2 (28.6)	

HP, high-grade polarized expression; LP, low-grade polarized expression; D, depolarized expression.

For adenocarcinoma patients, the difference in the postoperative survival between the three groups as defined with MUC1 status was enhanced. The 5-year survival rates of HP, LP, and D patients were 78.3%, 51.4%, and 40.0%, respectively, showing a significant difference among the three groups ($p = 0.039$ by the log rank test and $p = 0.012$ by the Wilcoxon test) (Figure 3); the p values between HP and D patients by the log rank test and the Wilcoxon test were 0.015 and 0.006, respectively (Figure 3). A multivariate analysis taking into account age, pathologic T factor, lymph node metastasis, and MUC1 expression status in adenocarcinoma patients showed that depolarized MUC1 expression was a significant and independent factor to predict a poor postoperative prognosis (relative risk to high-grade polarized MUC1 expression, 4.73; 95% confidence interval, 1.00–22.3; $p = 0.049$); lymph node metastasis was also a significant prognostic factor (relative risk, 5.81; 95% confidence interval, 1.94–17.4; **p** = 0.002).

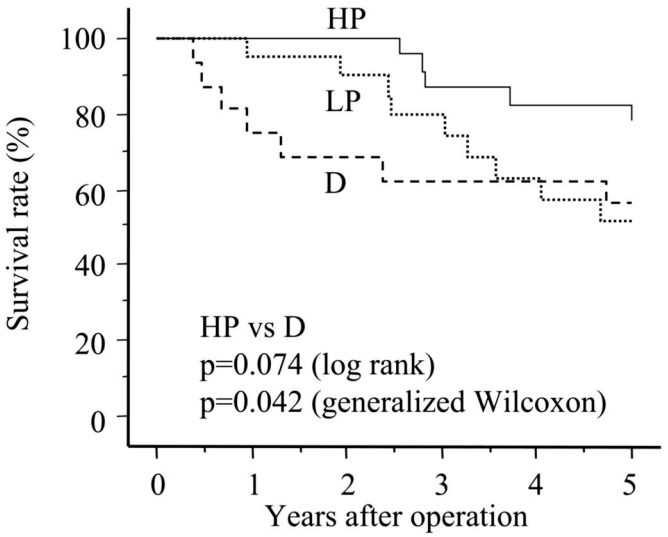

FIGURE 2. Postoperative survival of NSCLC patients according to MUC1 expression pattern. HP, high-grade polarized expression; LP, low-grade polarized expression; D, depolarized expression.

DISCUSSION

In the present study, we conducted a detailed analysis of MUC1 expression in NSCLC, and classified NSCLC patients into three groups according to MUC1 expression patterns: such as high-grade polarized, low-grade polarized,

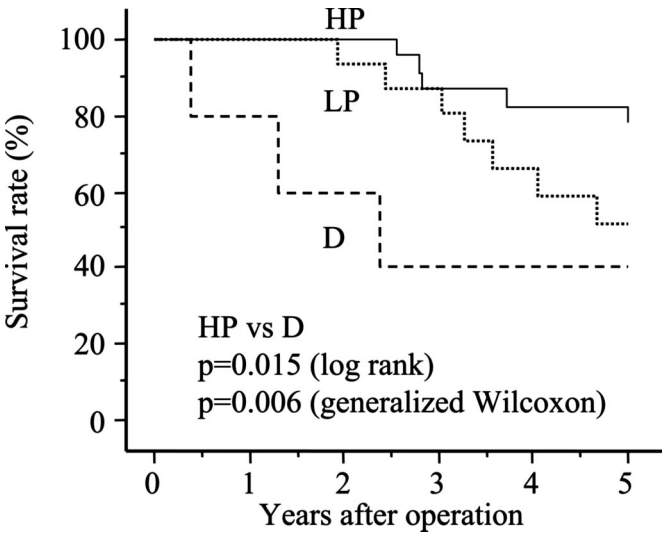

FIGURE 3. Postoperative survival of lung adenocarcinoma patients according to MUC1 expression pattern. HP, high-grade polarized expression; LP, low-grade polarized expression; D, depolarized expression.

and depolarized expression. Some studies had already demonstrated that enhanced depolarized MUC1 expression and down-regulated MUC1 expression was positively correlated with dedifferentiation of lung adenocarcinoma.[36,37] In the present study, HP expression was exclusively observed in adenocarcinoma patients and was mostly observed in well-differentiated adenocarcinoma patients; LP or D expression was, in contrast, mostly observed in moderately to poorly differentiated adenocarcinoma patients or in other histology patients. These results are consistent with previous studies,[36,37] and we propose the MUC1-expression classification as a simple and effective method for evaluation of tumor differentiation of lung adenocarcinoma.

We also assessed the prognostic significance of MUC1 expression pattern in resected NSCLC and showed that patients with depolarized MUC1 expression tended to show a poor prognosis after thoracotomy for NSCLC; in lung adenocarcinoma, depolarized expression proved to be a significant factor for predicting a poor prognosis. The Kaplan-Meier survival curves in the present study (Figures 2 and 3) showed that postoperative death in D patients might occur in relatively early phase of postoperative course as compared with that in HP patients. Many clinical studies have demonstrated that MUC1 status may be a useful marker of tumor progression and/or poor outcome in a variety of malignant tumors, including thyroid,[19] breast,[20] gastric,[21,22] pancreatic,[23] bile duct,[24,25] gallbladder,[26] ovarian,[27] uterus endometrial,[28] prostate,[29] and colorectal cancers[30]; results in the present study that depolarized expression was a predictor of a poor prognosis were inconsistent with these previous studies. In some studies, aberrant MUC1 expression on the stroma-facing cell surface (stromal pattern) or diffuse distribution in the cytoplasm (cytoplasmic pattern) was correlated with a poor prognosis.[25,26,30] In NSCLC, an expression pattern corresponding to such stromal or cytoplasmic pattern has been described as depolarized expression as defined in the present study, but prognostic significance of MUC1 status has not been established.[6,31,32] Jarrard et al. proposed that MUC1 is a marker of the type II pneumocyte lineage during carcinogenesis but showed that MUC1 status, positive or negative, was not correlated with survival.[6] Guddo et al. classified MUC1 expression patterns into positive depolarized or negative depolarized expression; positive depolarized MUC1 expression was correlated with poor survival in lung squamous cell carcinoma patients, but not in adenocarcinoma patients.[31] Tsutsumida et al. evaluated MUC1 expression in combination with surfactant apoprotein A (SP-A) expression in small-size lung adenocarcinoma; patients with tumors showing dominant MUC1 expression over SP-A expression (MUC1 > SP-A) showed a significant poor survival, whereas MUC1 status alone was not correlated with survival.[32] In the present study, we conducted a more detailed MUC1 status classification (HP, LP, and D), and showed a significant difference in postoperative survival between HP and D patients in lung adenocarcinoma.

Despite recent development of radiotherapy and chemotherapy such as molecular targeting agents, the prognosis of NSCLC patients with unresectable disease or postoperative recurrence remains unsatisfactory.[33] Now, immunotherapy has been developed as a new option for cancer therapy. MUC1 core protein may be a useful target molecule for immunotherapy in breast cancer and other malignancies expressing MUC1 including NSCLC.[18,38,39] Because antitumor effecter cells such as cytotoxic T lymphocytes may infiltrate into tumor parenchyma from stromal vessels around tumor,[40] a MUC1-targeted immunotherapy may be more appropriate for tumors showing depolarized MUC1 expression than those showing polarized expression on the ductal side of tumor cells. Correlation between the efficacy of a MUC1-targeted immunotherapy and MUC1-expression status should be investigated.

In conclusion, we proposed a novel classification of MUC1 expression pattern and showed that the classification was a useful marker correlated with postoperative survival as well as tumor differentiation in lung adenocarcinoma.

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